

WHAT IS CLAIMED IS:

1. A method of expressing a polynucleotide in a mammal, comprising:

administering viral particles comprising a recombinant adeno-associated virus (AAV) vector to liver cells of said mammal, wherein said recombinant AAV (rAAV) vector comprises said polynucleotide, such that said polynucleotide, or a portion thereof, is expressed in said mammal.

2. The method of claim 1, wherein said polynucleotide is expressed in vivo.

3. The method of claim 2, wherein said mammal is a human.

4. The method of claim 1, wherein liver cells of said mammal are transduced with said recombinant AAV vector ex vivo, and wherein said administering further comprises delivering said transduced liver cells into the portal vasculature of said mammal.

5. The method of claim 4, wherein said mammal is a human.

6. The method of claim 5, wherein said polynucleotide encodes a therapeutically effective polypeptide for treating a human disease or disorder.

7. The method of claim 1, wherein said polynucleotide of said rAAV vector comprises:

a promoter capable of expression in human liver cells,
a structural gene encoding a therapeutically effective polypeptide, and

two AAV Inverted Terminal Repeats, wherein said Inverted Terminal Repeats flank the promoter and structural gene.

8. The method of claim 7, wherein said Inverted Terminal

Repeats comprise a portion of said wild-type AAV Inverted Terminal Repeats.

9. The method of claim 7, wherein said promoter is obtained from a virus.

10. The method of claim 9, wherein said virus is murine leukemia virus.

11. The method of claim 10, wherein said virus is Moloney murine leukemia virus.

12. The method of claim 11, wherein said rAAV vector is rAAV-MFG-human Factor IX.

13. The method of claim 4, wherein said polynucleotide comprises an antisense polynucleotide.

14. The method of claim 4, wherein said polynucleotide encodes a ribozyme.

15. The method of claim 1, further comprising:
providing said mammal with a partial hepatectomy.

16. The method of claim 1, further comprising:
administering a helper virus into the portal vasculature of said mammal.

17. The method of claim 1, further comprising:
administering a secondary agent for enhancing transduction efficiency to said liver cells of said mammal.

18. The method of claim 17, wherein said administering of said secondary agent and said administering of said recombinant AAV vector occurs in vivo.

19. The method of claim 3, wherein a secondary agent is

applied to said liver cells of said mammal to enhance transduction with said recombinant AAV vector ex vivo.

20. The method of claim 1, wherein said administering further comprises:

injecting said recombinant AAV vector into the portal vasculature of said mammal.

21. The method of claim 20, wherein said mammal is a human.

22. The method of claim 21, wherein said polynucleotide encodes a therapeutically effective polypeptide for treating a human disease or disorder.

23. The method of claim 21, wherein said polynucleotide comprises an antisense polynucleotide.

24. The method of claim 21, wherein said polynucleotide encodes a ribozyme.

25. The method of claim 1, wherein a polypeptide encoded by said polynucleotide is expressed and detectable in an elevated level in blood of the mammal as compared to a level of said polypeptide in blood of said mammal prior to said administering step.

26. The method of claim 1, wherein said liver cell is a hepatocyte.

800 35 27. A method of treating a liver disease or disorder in a mammal, comprising:

administering a therapeutically effective dosage of a recombinant adeno-associated virus (AAV) vector to the liver cells of said mammal, wherein said recombinant AAV vector comprises a polynucleotide which encodes a product with a therapeutic effect on said disease or disorder.

28. The method of claim 27, wherein said polynucleotide encodes a therapeutically effective polypeptide for treating said liver disease or disorder.

29. The method of claim 27, wherein said polynucleotide encodes a ribozyme.

30. The method of claim 27, wherein said polynucleotide comprises an antisense polynucleotide.

31. A method of treating a disease or disorder in a mammal, comprising:

administering a therapeutically effective dosage of a recombinant adeno-associated (AAV) vector to the liver cells of said mammal, wherein said recombinant AAV vector comprises a polynucleotide operably linked to a promoter or enhancer that specifically functions in liver cells, wherein said polynucleotide encodes a product with a therapeutic effect on said disease or disorder.

32. The method of claim 31, wherein said liver cells are hepatocytes.

33. The method of claim 31, wherein said promoter is obtained from a virus.

34. The method of claim 33, wherein said virus is murine leukemia virus.

35. The method of claim 34, wherein said virus is Moloney murine leukemia virus.

36. A method of gene therapy for a mammal, comprising:

administering a therapeutically effective dosage of a recombinant adeno-associated (AAV) vector to the liver cells of said mammal, wherein said recombinant AAV vector comprises a polynucleotide which encodes a gene product with

~~a~~ therapeutic effect in said mammal.

37. The method of claim 36, further comprising:

identifying a therapeutically effective polypeptide encoded by said polynucleotide and

constructing a recombinant AAV vector comprising said polynucleotide operably linked to a promoter and a polyadenylation sequence.

38. The method of claim 37, wherein said mammal is a human.

39. The method of claim 36, wherein said liver cells are hepatocytes.

40. The method of claim 37, wherein said promoter is obtained from a virus.

41. The method of claim 40, wherein said virus is murine leukemia virus.

42. The method of claim 41, wherein said virus is Moloney murine leukemia virus.

43. A pharmaceutical composition for treating a human disorder comprising:

a recombinant adeno-associated (AAV) vector comprising a polynucleotide operably linked to a promoter and a polyadenylation sequence; and

a pharmaceutically acceptable carrier.

44. The composition of claim 43, wherein said promoter is obtained from a virus.

45. The composition of claim 44, wherein said virus is murine leukemia virus.

46. The composition of claim 45, wherein said virus is

Moloney murine leukemia virus.

47. The composition of claim 43, wherein said recombinant AAV vector is rAAV-MFG-huFIX.

48. A method for determining the presence of wild-type adeno-associated virus (AAV) and infectious AAV generated by recombination of a helper AAV and a vector AAV containing a transgene in a sample of recombinant AAV, wherein said vector AAV contains nucleotide sequences or has an order of nucleotide sequences different from that of wild-type AAV, comprising:

- a) obtaining nucleic acids from AAV contained in said sample;
- b) performing a nucleic acid amplification reaction on said nucleic acids using oligonucleotides specific for wild-type AAV and specific for infectious AAV generated by recombination; and
- c) determining the presence of wild-type AAV and infectious AAV generated by recombination by the presence of amplified nucleic acids specific thereto in said amplification reaction.

49. The method of claim 48, wherein said amplification reaction is a polymerase chain reaction.

50. The method of claim 48, wherein said vector AAV is pSUB201 or a derivative thereof containing AAV right end nucleotide sequences on the left end of said vector and lacking AAV left end nucleotide sequences.

51. The method of claim 49, wherein said method comprises two polymerase chain reactions, wherein a first reaction comprises a first primer which hybridizes to wild-type AAV and to wild-type AAV generated by recombination and a second primer which hybridizes to wild-type AAV generated by recombination; and a second reaction comprises said first

primer and a third primer which hybridizes to wild-type AAV.

52. The method of claim 51, wherein said second primer and said third primer hybridize to the left end of said AAV nucleic acids.

53. The method of claim 52, wherein said second primer is D2 and said third primer is D1.

54. A kit comprising:

- (a) an oligonucleotide which hybridizes to wild-type adeno-associated virus (AAV); and
- (b) an oligonucleotide which hybridizes to wild-type AAV generated by recombination.

55. The kit of claim 54, further comprising:

- (c) an oligonucleotide which hybridizes to wild-type AAV and to wild-type AAV generated by recombination.

56. The kit of claim 54, wherein said oligonucleotides (a) and (b) further comprise at the 5' end thereof an RNA polymerase promoter.